

To Determine the Plasma Levels of Malondialdehyde in Patient with Pre-eclampsia and Healthy Pregnant Women of Hyderabad, Sindh

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ABSTRACT

OBJECTIVES: Pre-eclampsia is leading cause of maternal mortality and morbidity throughout the world. In Pakistan the mortality rate of pregnancy related problems is estimated to be approximately 500 per 100,000 live births. According to several studies oxidative stress and production of free radicles may be responsible for abnormal development of placenta results in the pathogenesis of pre-eclampsia. During oxidative stress Malondialdehyde has been proposed as novel factors concerned in development of pre-eclampsia.

METHOD: Total 210 voluntaries were taken for the study, from which 50 voluntaries were normotensive healthy pregnant women as a control, 160 were patient with pre-eclampsia as cases. The subjects were recruited after taking obstetric history, anthropometric and clinical data. After taking informed consent, blood sample collected and stored for biochemical analysis. Malondialdehyde was analyzed on spectrophotometer. The study was conducted in Biochemistry Department Liaquat University of Medical & Health Sciences, Jamshoro from February 2015 to October 2015.

RESULTS: Two hundred and ten subjects were included in this study. The Mean \pm S.D of age of controls and pre-eclamptic subjects were noted as 27.5 ± 4.5 and 27.38 ± 4.3 years. Gestational ages of controls were 25.76 ± 4.35 and cases were noted as 27.37 ± 4.49 ($p < 0.053$). The mean difference of serum Malondialdehyde levels in controls were 1.54 ± 0.28 nmol/dl, while in pre-eclamptic subjects it was 2.42 ± 0.63 nmol/dl which is highly significant and $p < 0.05$ respectively.

CONCLUSION: Malondialdehyde is index marker of lipid peroxidation. Increased formation of free radicles due to increased concentration of Malondialdehyde may put forward the possible involvement in the pathology of PE. The aim of this study to protects the mothers as well as her coming newborn from such life threatening disease.

KEYWORDS: Malondialdehyde, Lipid peroxidation, Pre-eclampsia (PE), Uv-Spectrophotometer

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INTRODUCTION

Pre-eclampsia (PE) is a pregnancy related complication occurs after the 20th week of pregnancy with systolic blood pressure of 140 mm of Hg or diastolic blood pressure of 90 mmHg or more, taking two readings six hours apart accompanied by significant proteinuria 300 mg or more in 24 hours urine collection^{1, 22}. According to World Health Organization (WHO) the incidence of PE in 2014 was 19% in developing countries, 35% in Pakistan, 33% in India, 28% in Africa and 23% in South America²¹. Pathogenesis of PE and its causative factors are still unclear. Many researchers proposed that it is developed due to endothelial cell injury, which produced oxidative species resulting in uncontrolled lipid peroxidation (LPO)². Endothelial dysfunction also

leads to cause impairment of nitric oxide pathway, causes reduced blood flow and poor placentation¹³. Oxidative stress is an imbalance between production of free radicals and antioxidants which leads to cause potential damage to the cell. In normal condition free radicals and antioxidants are in balance¹². Oxidative stress is increased throughout pre-eclampsia, resulting in enhanced production of lipid peroxides²². Metabolic changes and inflammation increases the oxidative stress by producing reactive oxygen species and Nitric oxide radicals¹³. Malondialdehyde (MDA) is an indicator of lipid peroxidation (LPO). MDA is three carbon aldehyde and break down products, produces free radicals which act on polyunsaturated fatty acid of lipid bilayer³. Lipid peroxidation is harmful to the cell

membrane because it acts as self-perpetuating reaction, Lipid peroxidation decreases the membrane potential by loss of fluidity and increased permeability to calcium ions and protons, which break down cell membrane⁶. Malondialdehyde is increased in many diseases but greatly increased in pregnancy-induced hypertension. Oxidative stress can produce interrelated derangements of cell metabolism. There are three phases of lipid peroxidation Initiation, propagation and termination⁷.

The end products of lipid peroxidation are reactive aldehydes, such as MDA and 4-hydroxynonenal (HNE). Lipid peroxidation it self amplifies for the formation of free radicals, single initiation event can results in conversion of hundreds of fatty acid side chains into lipid hydroperoxides^{8,10}. When MDA level increases, produce free radicals which damages the cell membrane and also have strong association with severity of disease²⁰. MDA also effect on DNA and causes oxidative stress by production of free radicals. The normal levels of Plasma MDA is 1.40-1.90 nmol dl, MDA levels increases throughout pregnancy but more in pre-eclampsia¹⁴.

PURPOSE OF STUDY

This study was conducted to evaluate the plasma levels of MDA in healthy pregnant women and compare with patients of pre-eclampsia, thus present study may be helpful to early diagnosis to pre-eclampsia.

OBJECTIVE OF STUDY

- To estimate the plasma levels of Malondialdehyde in healthy pregnant women and patient with pre-eclampsia.
- To compare the plasma levels of Malondialdehyde in healthy pregnant women and patient with pre-eclampsia.

SETTING

The study is conducted in Biochemistry Department, Liaquat University of Medical & Health Sciences, Jamshoro and patients were recruited from Department of Obstetrics & Gynecology, Liaquat University Hospital Jamshoro/Hyderabad.

SAMPLE SIZE

The sample size calculation was done according to general calculation formula. Pre-eclampsia²² is 5% by using the proportion of 95% confidential interval and 5% margin of error, the sample size calculated was n = 80.

Total 210 subjects were recruited and divided into two groups.

Group A: n = 50 Healthy pregnant women with normal B.P after 20 weeks of gestation and Group B: n = 160 patients with pre-eclampsia after 20 weeks of gestation

STUDY DESIGN: Cross-sectional, Comparative study.

STUDY DURATION: Within eight months after approval of study from Ethical Review Committee of LUMHS Jamshoro.

INCLUSION CRITERIA: Healthy pregnant women after 20 weeks of gestation. Pregnant women with pre-eclampsia after 20 weeks of gestation, age in between 20-35 years.

EXCLUSION CRITERIA: Pregnant women below 20 or above 35 years, Multiple Pregnancy Known hypertensive, diabetic, cardiac and renal patients, any liver disease, Smokers. Any use of drug.

METHODOLOGY

The study comprised on two phases. Phase 1 was recruitment of volunteers and phase 2 was collecting blood samples from the volunteers with 12-14 hours fasting. Population informed for the recruitment by the flyers at Department of Obstetrics and Gynecology, Jamshoro/Hyderabad.

BLOOD PARAMETERS

About 03 ml of blood was collected from each participant by venopuncture into vacutainers under aseptic measures, collected into EDTA test tube for MDA. The blood was centrifuged at 3500 rpm for 5 min by centrifuged machine; the plasma was fractionated and transferred to eppendorf cups then stored at -20°C till required for analysis. Before the analysis sample was first allowed to attain room temperature then used.

DATA COLLECTION

For this study, all clinical data and relevant details of each participant was registered by filling a Performa or Questionnaire. Verbal and written consent was taken from all participants, explaining them about study purpose.

ETHICAL CONSIDERATION

The study was conducted strictly under the ethical rules after the approval from Ethical Review Committee of LUMHS Jamshoro.

STATISTICAL ANALYSIS PROCEDURE

Data was entered in Microsoft Excel and analyzed on SPSS (Statistical package for Social Sciences) Version 16. Student t-test for comparison between cases and controls were used for continuous variables. Results were presented as mean and standard deviation.

EXPERIMENTAL PROTOCOL: Malondialdehyde

procedure was performed on spectrophotometer UV 1650 PC SHIMADZU.

ESTIMATION OF MALONDIALDEHYDE: Chemicals and Reagents such as, methanol

Trichloroacetic acid (TCA), Orthophosphoric acid (H_3PO_4), Thiobarbituric acid (TBA), Sulfuric acid (H_2SO_4) and Standard of MDA 1, 1, 3, 3- Tetraethoxypropane (TEP). All chemicals and reagents were of analytical grade and purchased from Sigma-Aldrich.

STANDARD PREPARATION: 1, 1, 3, 3- Tetraethoxypropane (TEP) was used as standard. For the preparation of stock solution, taken 25 μ l TEP dissolved in 100 ml of distilled water to give 1mM stock solution. Working standard was prepared by hydrolysis of 1 ml TEP stock solution in 50 ml 1% sulfuric acid, left at room temperature for two hours, protected the mixture from the light and keeps it at $-20^\circ C$ for 18 months.

To determine the different concentration of standard used 0 as blank (distilled water), standard of 5, 10 and 20 nmol/ml TEP were used.

ESTIMATION OF MDA BY THIOBARBITURIC ACID METHOD

PROCEDURE

100 μ l of plasma and 25 μ l 3 M NaOH was taken in test tube. The tubes were capped, vortexed and placed in water bath at $60^\circ C$ for 30 minutes. For the hydrolysis, 1 ml of sulfuric acid and 0.5 ml 20% TCA were added then centrifuged at 3000 rpm for 5 minutes then added 1ml of TBA in the test tube. 1 ml of supernatant layer was removed and again kept in water bath for 30 minutes. Upon cooling the mixture rapidly centrifuged at 3000 rpm for 10 minutes and analyzed for Malondialdehyde levels on UV-spectrophotometer at 532nm^{14, 15}.

RESULTS

The results of present study was carried out to evaluate the significance of plasma Malondialdehyde in pre-eclampsia and the results are taken as (mean \pm SD) summarized in Table-I and presented in graph-I. Total 210 subjects were included in this study, 50 were normotensive pregnant women and 160 were patients with pre-eclampsia. Maternal age of normal pregnant healthy women was (26.5 \pm 4.28) weeks and pre-eclamptic subjects were (26.38 \pm 5.16) weeks respectively. Gestational ages of controls and cases were (28.76 \pm 4.48) and (27.37 \pm 4.49) years respectively which are insignificant ($p < 0.05$) as shown in table I & presented in graph I.

Systolic blood pressure (mean \pm SD) in controls were noted as (113.2 \pm 9.13) mmHg, while in pre-eclamptic

it was (175.9 \pm 2.59)mmHg showing statistically significant. Diastolic B.P in controls were noted as (74.40 \pm 6.75) mmHg and pre-eclamptic subjects (110.5 \pm 9.75)mmHg was highly significant ($p < 0.05$) as shown in table-1 and presented in graph 1 respectively.

The Plasma Malondialdehyde levels (mean \pm SD) in controls were (1.54 \pm 0.28)nmol/d and in pre-eclamptic subjects (2.42 \pm 0.63) nmol/dl. The plasma Malondialdehyde levels were significantly higher ($p < 0.05$) in pre-eclamptic patients as against the control subjects, values were shown in Table I and graphically represented in graph II.

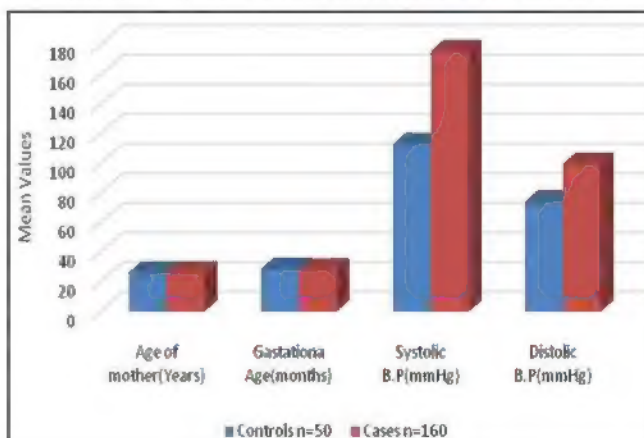
TABLE I: COMPARISON OF AGE, GESTATIONAL AGE, SYSTOLIC, DIASTOLIC BLOOD PRESSURE AND MALONDIALDEHYDE BETWEEN CONTROLS AND CASES

VARIABLES	Control (50)	Cases (160)	p- Value
	Mean \pm S.D	Mean \pm S.D	
Age (years)	26.5 \pm 4.28	26.38 \pm 5.16	$\leq 0.05^*$
Gestational Age (Weeks)	28.76 \pm 4.48	27.37 \pm 4.45	$\leq 0.05^*$
Systolic B.P (mmHg)	113.2 \pm 9.13	175.9 \pm 2.59	$\leq 0.05^*$
Diastolic B.P (mmHg)	74.40 \pm 6.75	110.5 \pm 9.75	$\leq 0.05^*$
MDA (nmol/l)	1.54 \pm 0.28	2.42 \pm 0.63	$\leq 0.05^*$

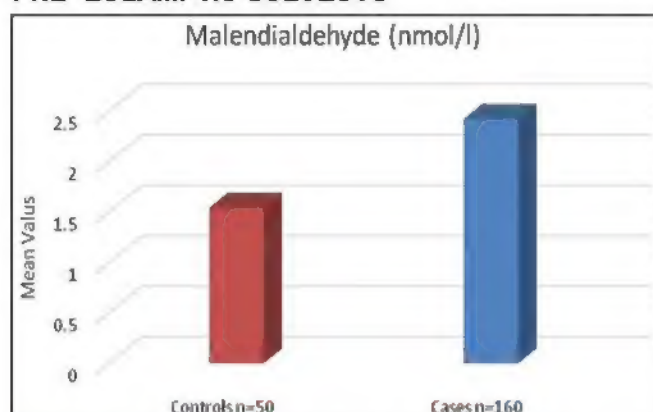
The values are expressed as mean \pm standard deviation and units are given in parenthesis. Results are presented as p values and calculated by student t-test * $p < 0.05$, ** $p < 0.01$

GRAPH I:

COMPARISON OF MATERNAL AGE, GESTATIONAL AGE, SYSTOLIC AND DIASTOLIC BLOOD PRESSURE BETWEEN CONTROLS AND CASES



GRAPH II: COMPARISON OF MALONDIALDEHYDE BETWEEN HEALTHY PREGNANT WOMEN WITH PRE- ECLAMPTIC SUBJECTS



DISCUSSION

The purpose of this study was to evaluate the role of Malondialdehyde, an oxidative stress marker in patients with pre-eclampsia. Many studies had been reported on the Malondialdehyde levels in pre-eclamptic women¹⁷. The incidence of pre-eclampsia is rising in developing countries day by day and pregnant ladies have been suffering by this life threatening disease^{9,11}. Specific marker of oxidative stress is Malondialdehyde. It is ultimate product of oxidative disintegration of polyunsaturated fatty acids and is commonly considered as oxidative stress biomarker¹⁸. Aksoy H *et al.*³ had reported that Malondialdehyde levels were increased in patients of pre-eclampsia which lead to cause oxidative stress due to increased production of free radicals. In present study results shows that oxidative stress occurs due to increased levels of Malondialdehyde which effect on cellular functions.

Saxena S *et al.*¹⁹ had reported strong and significant relationship between Malondialdehyde and blood pressure. Malondialdehyde effects on blood pressure due to increased generation of oxygen radical species and reduction in antioxidants. In our study plasma Malondialdehyde levels increased significantly in pre-eclamptic women as compared to control subjects.

Atamer Y *et al.*⁴ had reported that normally free radicals and antioxidants are in balance but in pre-eclampsia this balance distressed which causes oxidative stress resulting in enhance production of free radicals causes excessive consumption of antioxidants, which includes vitamins A, ascorbic acid, trocopherol and antioxidants enzymes such as glutathione peroxidase and superoxide dismutase⁵. These findings are supporting the present study as well as we observed similar imbalance effects of oxidative stress and antioxidants (by analyzed MDA)

in patient with pre-eclampsia.

Gohil JT *et al.*⁹ observed that during normal pregnancy Malondialdehyde levels were increased and gradually more increased in pre-eclampsia patients as compare to non-pregnant. In our study it was found that Malondialdehyde levels in healthy pregnant women are in normal but significantly increase in pre-eclamptic patients.

Rafeenia A *et al.*¹⁶ had reported that an increase levels of Malondialdehyde in patient with pre-eclampsia is feature of uncontrolled oxidative stress which contribute in development of hypertension in pregnancy. This study is favoring the present study that MDA is increasing in pre-eclamptic patients and may play a role in pathophysiology of pre-eclampsia.

CONCLUSION

The present study showed increased Malondialdehyde levels in pre-eclamptic patient as compared to the normal healthy pregnant women. The oxidative stress increases and present study analyzed by index marker of lipid peroxidation such as Malondialdehyde. MDA can be used as biomarker for early diagnosis of preeclampsia.

RECOMMENDATION

Further studies are recommended to confirm the findings of present study and also emphasis other factors of lipid peroxidation which may causes the preeclampsia.

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